The response of cyclic 3′,5′-AMP and cyclic 3′,5′-GMP phosphodiesterases to experimental diabetes[1]

B. Perez de Gracia, A.R. Rhoads and W.L. West

Departments of Pharmacology and Biochemistry, Howard University, College of Medicine, Washington (D.C. 20059, USA), 4 October 1979

Summary. Alloxan diabetes caused a decrease in cyclic AMP phosphodiesterase in all affected rat tissues. Cyclic GMP phosphodiesterase activity was, however, decreased in adipose and liver, but increased in heart and uterus.

Previous investigators have demonstrated that insulin invokes changes in the activity of cyclic AMP phosphodiesterase (EC 3.1.4.17) in several tissues of rat[2-6]. With insulin-treated adipocytes, the increase in phosphodiesterase activity appears to be due to activation of a particulate, low K_m cyclic AMP phosphodiesterase[5,7]. In agreement with this insulin-activating effect, decreased cyclic AMP phosphodiesterase was previously observed in heart, liver, uterus and adipose tissue of alloxan-diabetic rats[2]. Since mammalian tissues contain multiple forms of cyclic 3′,5′-nucleotide phosphodiesterase[8,9] which appear to be under separate genetic control[10], we investigated the individual responses of cyclic AMP and cyclic GMP phosphodiesterase to insulin deprivation in alloxan-diabetic rats.

Materials and methods. The procedure of Londesbrough[11] was used in the assay of cyclic AMP and cyclic GMP phosphodiesterases. The reaction mixture contained 40 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$ and 1 µM cyclic AMP or cyclic GMP. Purified tritiated cyclic nucleotide (200,000 cpm) was added to each 0.5-ml reaction mixture. After incubation at 30 °C for 10 min, the reaction was terminated by heating at 100 °C for 2 min and the mixture incubated for an additional 10 min with 100 µg of *Crotalus atrox* venom. After addition of the anion exchange resin, the suspension was centrifuged and a 0.5-ml aliquot of the supernatant was counted in 5 ml of Hydromix (Yorktown Research, Hackensack, N.J.) with an absolute efficiency of 41%. A µunit of enzyme activity is defined as the production of 1 pmole of product/min. Specific activity was expressed as µunits/mg of tissue protein as determined by Lowry et al.[12].

Each 100 mg of tissue was homogenized in 1 ml of 0.33 M sucrose-containing 0.04 M Tris-HCl and 0.005 M $MgCl_2$ adjusted to pH 7.4. Homogenization was considered complete after 5 strokes with a Ten-Broeck glass homogenizer. The whole homogenate was used in the assay of cycli AMP and cyclic GMP phosphodiesterase.

Diabetic animals were produced by injection of alloxan into the tail vein of female Sprague-Dawley rats. After 24–48 h, blood glucose was determined and only those animals in which blood glucose exceeded 200 mg% were included in the diabetic group. Controls were treated with 0.9% NaCl. Renal glucosuria was assessed by the Hema-Combistix test (Ames Co., Elkhart, Indiana).

Results. The activity of cyclic AMP and cyclic GMP phosphodiesterase in cell-free tissue homogenates of alloxan-treated and control rats is shown in the table. In those tissues where the enzyme activity was found to be altered, both cyclic AMP and cyclic GMP phosphodiesterase were effected simultaneously. Cyclic AMP phosphodiesterase activity was always decreased in response to insulin deficiency. Decreased cyclic AMP phosphodiesterase activity was observed in heart, liver, uterus and adipose tissues of diabetic rats. No significant change in enzyme activity occurred in the brain cortex, diaphragm, kidney, or gastrocnemius muscle. These results are in good agreement with earlier investigations of cyclic AMP phosphodiesterase[2] which demonstrated decreased cyclic AMP phosphodiesterase activity in liver and adipose tissue of diabetic rat and no change occurring in kidney or skeletal muscle of these same animals. These findings also correlate well with the demonstrated in vitro activation of cyclic AMP phosphodiesterase of adipocytes[3,5-7] and hepatocytes[13] by insulin.

In the table, cyclic GMP phosphodiesterase activity of heart, liver, uterus and adipose tissue of diabetic rats was significantly different from control tissues. Unlike cyclic AMP phosphodiesterase, cyclic GMP phosphodiesterase increased in some tissues and decreased in others in response to experimentally induced diabetes. Cyclic GMP phosphodiesterase activity decreased in parallel with cyclic AMP phosphodiesterase activity in liver and adipose tissue. In heart and uterus, cyclic GMP phosphodiesterase activity was activated in response to alloxan diabetes while cyclic AMP phosphodiesterase was inversely effected.

Discussion. Only a few investigations have examined the hormonal response of both phosphodiesterase activities simultaneously. In agreement with these studies, Correze et al.[14] found parallel increases in both cyclic AMP and cyclic GMP phosphodiesterase activity in adipose tissue after insulin exposure. However, Das and Chain[15] reported parallel decreases in both cardiac phosphodiesterases activities in streptozotocin-treated rats. The latter study, however, assayed phosphodiesterase activities at high, unphysiological concentrations of cyclic nucleotides which would measure only the predominant enzyme with the significantly higher maximum velocity. In this study, phosphodiesterase activity was assayed by initial velocity measurement at 1 µM.

Cyclic 3′,5′-nucleotide phosphodiesterase activities of different tissues from normal and alloxan-treated rats

Tissue	Cyclic AMP phosphodiesterase			Cyclic GMP phosphodiesterase		
	Control	Alloxan-treated	% change	Control	Alloxan-treated	% change
Brain cortex	414±21 (4)	421±26 (6)	NS	538±25 (4)	514±38 (4)	NS
Heart	49.1±2.8 (8)	32.5±2.4 (6)	−33.8[a]	47.2±0.70 (4)	64.1±0.93 (5)	+35.8[a]
Diaphragm	27.7±1.3 (9)	31.4±1.5 (6)	NS	27.8±1.9 (9)	26.8±1.6 (5)	NS
Liver	39.6±2.3 (9)	30.7±0.80 (6)	−22.5[b]	67.4±4.0 (9)	35.7±2.8 (6)	−47.0[a]
Kidney	136±14 (4)	137±7.0 (4)	NS	146±7.4 (4)	134±6.1 (4)	NS
Uterus	62.2±4.4 (9)	48.4±2.2 (6)	−22.2[b]	112±4.3 (9)	155±7.7 (6)	+38.4[a]
Adipose	23.3±1.5 (9)	13.2±0.20 (6)	−43.3[a]	38.3±3.9 (9)	20.6±2.9 (6)	−46.2
Gastrocnemius	30.7±1.9 (4)	30.2±0.37 (4)	NS	22.5±1.4 (4)	20.1±1.8 (4)	NS

Phosphodiesterase activity is expressed as µunit/mg protein ± SD. Numbers in parentheses represent the numbers of animals. Tissue homogenates were assayed at 1 µM cyclic nucleotide. Rats were injected i.v. with alloxan (60 mg/kg) to induce diabetes. P was calculated by Student's t-test by comparison with control values; [a] $p < 0.001$; [b] $p < 0.01$.

In summary, we find that cyclic AMP phosphodiesterase activity always results in a decreased activity in specific tissues of alloxan-diabetic rats, whereas cyclic GMP phosphodiesterase may be increased or decreased in activity. These results support the view that separate enzymes with discrete regulation are responsible for the degradation of cyclic AMP and cyclic GMP in certain mammalian tissues and that the action of insulin on different tissues is highly specific.

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A comparison of 3 reagents in converting thiamine to thiochrome in the presence of plant extracts and polyphenols

K. Ratanaubolchai, S. Pikulkarntalert and B. Panijpan[1]

Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok (Thailand), 8 October 1979

Summary. Assays of thiamine added to plant extracts, known polyphenols and reducing agents, using $K_3Fe(CN)_6$, $HgCl_2$ and CNBr showed that the CNBr method was the least susceptible to redox interference and gave the highest thiochrome yield.

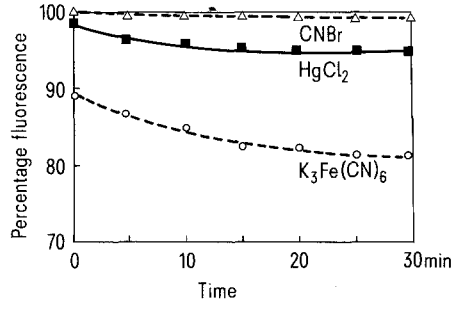
As a result of our research into the thiamine-modifying ability of polyphenolic compounds, we have found the widely used potassium ferricyanide reagent to be particularly susceptible to redox interference by polyphenols[2,3]. This kind of interference is a serious yet not fully appreciated problem caused by compounds, capable of oxidation-reduction, that are not significantly or easily extracted into the isobutanol organic solvent layer, and thus do not interfere with the thiochrome fluorescence as such. The lack of proper appreciation is exemplified by a statement about the ferricyanide assay 'If the sample extracts give low (fluorescence) blanks, the Decalso step may be omitted –'[4]. Since we have good reason to doubt the general validity of the statement[2,3], we have been testing 2 other reagents, $HgCl_2$ and CNBr for their liability to interference by polyphenols and other compounds. Thiamine added to plant extracts (usually rich in polyphenols) and known polyphenols has been assayed using $K_3Fe(CN)_6$, $HgCl_2$ and CNBr and the thiochrome fluorescence in the isobutanol extract compared with that produced from thiamine alone.

Materials and methods. Plant materials were treated and extracted as specified in the AOAC Handbook[5]. Some extracts were diluted before the assay to make them more manageable and freer from interfering substances. Polyphenols and reducing agents of good grade were purchased. The reagents ($K_3Fe(CN)_6$, $HgCl_2$ and CNBr) were prepared and used as described in the appropriate recipes[4,6,7]. Pre-extraction was done by twice vortexing an equal volume of isobutanol with the slightly acidified thiamine and/or polyphenol-containing solutions for 5 min. Fluorescence measurements were performed on an Amino Bowman spectro-

Table 1. Percentage relative fluorescence of added thiamine in plant extracts as developed by $K_3Fe(CN)_6$, $HgCl_2$ and CNBr. The standard errors vary from about 1 to 5%

Reagent	$K_3Fe(CN)_6$		$HgCl_2$		CNBr	
Treatment	I	II	I	II	I	II
Kapok seeds	92	97	51	52	91	94
Betel nut kernel	3.6	19	1.9	4.0	73	94
Betel leaves	n.d.	10	n.d.	44	n.d.	97
Peanut	78	96	60	72	101	99
Tomato	23	85	12	36	43	91
Lemon	1.4	92	3.8	64	63	94
N. oleracea, Lour	12	14	1.1	12	60	94
Rice bran	20	24	22	9.6	85	91

n.d. = not determinable because the extract became too viscous; I = plant extracts, II = plant extracts after pre-extraction by isobutanol.



Percentage relative fluorescence readings of thiochrome from eluates of the loaded tea infusion (1 g/50 ml) kept at 85 °C for various times. Here 1.25 ml of the infusion was loaded on to the Decalso column and 5 ml of the eluate was used for assay by the 3 reagents. If only 0.25 ml of the brew was used for column loading all 3 assays yielded the CNBr curve.